

IN THE CLAIMS

1. (currently amended) A method of detecting a target base sequence, comprising the steps of:

amplifying DNA containing a target base sequence to be detected having a mutation site using DNA polymerase;

hybridizing the amplified DNA to a hybridization probe with length of 10 - 15 mer having a base sequence complementary to the target base sequence to be detected; and

detecting a hybrid formed by the hybridization,

wherein,

at least one of primers to be used in the DNA amplification is labeled with a first labeling agent so that the amplified DNA will be labeled with the first labeling agent,

the hybridization probe is labeled with a second labeling agent and contained in a reaction solution for effecting the DNA amplification,

the hybridization probe has a Tm of 25°C to 40°C lower than the Tm of primers used in the DNA amplification;

the base sequence of the hybridization probe is designed not to inhibit the DNA amplification, and

the hybrid is detected by affinity chromatography with the use of the first and second labeling agents.

2. (original) The method according to claim 1, wherein the mutation site is a point mutation, and the reaction solution for effecting the DNA amplification further contains an unlabeled oligonucleotide having a base sequence different in a single base at the position of the point mutation from the base sequence of the labeled hybridization probe, in an amount sufficient to enhance the specificity of hybridization of the amplified DNA to the hybridization probe.

3. (original) The method according to claim 1 or 2, wherein the DNA amplification is carried out by PCR.

4. (currently amended) A kit comprising:

primers for amplifying DNA containing a target base sequence to be detected having a mutation site using DNA polymerase;

a hybridization probe with length of 10 - 15 mer having a base sequence complementary to the target base sequence to be detected; and

a test strip for affinity chromatography,

wherein<sub>1</sub>

at least one of the primers to be used in the DNA amplification is labeled with a first labeling agent so that the amplified DNA will be labeled with the first labeling agent,

the hybridization probe is labeled with a second labeling agent,

the hybridization probe has a Tm of 25°C to 40°C lower than the Tm of primers for amplifying DNA.

the base sequence of the hybridization probe is designed not to inhibit the DNA amplification, and

the test strip allows ~~ef~~ detection of a hybrid of the amplified DNA and the hybridization probe with the use of the first and second labeling agents.

5. (original) The kit according to claim 4, wherein the mutation site is a point mutation and the kit further comprises an unlabeled oligonucleotide having a base sequence different in a single base at the position of the point mutation from the base sequence of the labeled hybridization probe.

6. (original) The kit according to claim 4 or 5, wherein the primers are primers for PCR.

7. (previously presented) The method according to claim 1 wherein the length of the hybridization probe is a 10 - 13 mer.

8. (previously presented) The method according to claim 7 wherein the length of the hybridization probe is a 11 - 13 mer.

9. (previously presented) The method according to claim 8 wherein the length of the hybridization probe is a 12 mer or a 13 mer.

10. (currently amended) The method according to claim 1 wherein the length of the hybridization probe is selected so that hybridization to the target base sequence does not substantially occur at a temperature at which the DNA polymerase is actively amplifying the target base sequence.

11. (canceled)

12. (currently amended) The method according to claim 1 wherein the Tm of the hybridization probe is 30 to 35°C lower than the Tm of the at least one labeled primer primers used in the DNA amplification.

13. (previously presented) The method according to claim 1 wherein the mutation site is an insertion mutation.

14. (previously presented) The method according to claim 1 wherein the mutation site is a deletion mutation.

15. (currently amended) The method according to claim 1 wherein the hybridization probe contains a sequence complimentary to the mutation site of the target base sequence at about a middle of the hybridization probe.

16. (currently amended) A method of detecting a target base sequence, comprising the steps of:

amplifying DNA containing a target base sequence to be detected having a mutation site using DNA polymerase;

hybridizing the amplified DNA to a 12 mer or 13 mer hybridization probe having a base sequence complementary to the target base sequence to be detected; and

detecting a hybrid formed by the hybridization,  
wherein,

the mutation site of the target base sequence is selected from the group consisting of a point mutation, an insertion mutation, and a deletion mutation;

at least one primer to be used in the DNA amplification is labeled with a first labeling agent so that the amplified DNA will be labeled with the first labeling agent;

the hybridization probe is labeled with a second labeling agent and contained in a reaction solution for effecting the DNA amplification;

the base sequence of the hybridization probe is designed not to inhibit the DNA amplification;

hybridization to the target base sequence does not substantially occur at a temperature at which the DNA polymerase is actively amplifying the target base sequence;

the Tm of the hybridization probe is 30 to 35°C lower than the Tm of the at least one labeled primer; and

the hybrid is detected by affinity chromatography with the use of the first and second labeling agents.

17. (previously presented) The method according to claim 16 wherein the mutation site is a point mutation, and the reaction solution for effecting the DNA amplification further contains an unlabeled oligonucleotide having a base sequence different in a single base at the position of the point mutation from the base sequence of the labeled hybridization probe, in an amount sufficient to enhance the specificity of hybridization of the amplified DNA to the hybridization probe.

18. (new) The method according to claim 1 wherein hybridizing the amplified DNA to the hybridization probe occurs at 25°C.

19. (new) The method according to claim 1 wherein the hybridization probe is a 10 mer.

20. (new) The method according to claim 1 wherein the hybridization probe is a 11 mer.